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Report on the Verification of the Performance of MON 87427, MON 89034, 1507, MON 88017 and 59122 event-specific PCR-based Methods applied to DNA extracted from GM Stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize

European Union Reference Laboratory for
Genetically Modified Food and Feed

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European Union Reference Laboratory for GM Food & Feed

Report on the Verification of the Performance of MON 87427, MON 89034, 1507, MON 88017 and 59122 event-specific PCR-based Methods applied to DNA extracted from GM Stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize

3/8/2017

European Union Reference Laboratory for GM Food and Feed

Executive Summary

An application was submitted by Monsanto Company, represented by Monsanto Europe S.A., to request the authorisation of genetically modified stack (GM stack) MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize [lepidopterian and coleopteran (corn rootworm) protection traits and glufosinate and glyphosate tolerance] and all sub-combinations of the individual events as present in the segregating progeny, for food and feed uses, import and processing, in accordance with articles 5 and 17 of Regulation (EC) N° 1829/2003 GM Food and GM Feed. The unique identifier assigned to GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize is MON-87427-7 x MON-89Ø34-3 x DAS-Ø15Ø7-1 x MON-88Ø17-3 x DAS-59122-7.

The GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize was obtained by conventional crossing between the genetically modified maize events: MON 87427, MON 89034, 1507, MON 88017 and 59122, without any new genetic modification.

The EURL GMFF has previously validated individually, and declared fit for purpose, the detection methods for the single events MON 87427, MON 89034, 1507, MON 88017 and 59122 (see http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx). In line with the approach defined by the ENGL (http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf), the EURL GMFF carried out an *in-house* verification of the performance of each validated method when applied to genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

The results of the *in-house* verification led to the conclusion that the individual methods meet the ENGL performance criteria also when applied to genomic DNA extracted from the GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

This report is published at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx.

Content

Quality assurance

The EURL GMFF is ISO 17025:2005 accredited [certificate number: ACCREDIA 1172 (Flexible Scope for DNA extraction and qualitative/quantitative PCR) - Accredited tests are available at http://www.accredia.it/accredia_labsearch.jsp?ID_LINK=293&area=7] and ISO 17043:2010 accredited (certificate number: ACCREDIA 0012, proficiency test provider).

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1. Introduction

The EU legislative system [1, 2] for genetically modified food and feed forsees that any GMO for food and feed use shall undergo the authorisation process before it can be placed on the market. This holds true also for a GMO containing more than one single GM event obtained by conventional crossing, co-transformation or re-transformation (genetically modified stack).

Consequently, the application for authorisation of a GM stack shall be accompanied, among others, by an event-specific method for detection, identification and quantification for each GM event composing the stack, and by samples of the stack and food and feed derived from it. The EURL GMFF shall validate the event specific methods of detection proposed by the applicant with regard to their performance when applied to DNA extracted from the stack, and shall report to the European Food Safety Authority, who will include the EURL GMFF report in the overall opinion concerning the risk assessment and potential authorisation of the assessed stack. In line with the approach defined by the ENGL (http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf) the EURL GMFF carries out an *in-house* verification of the performance of each event-specific methods if this method has previously been validated by the EURL GMFF for the parental single-line event and these events have been stacked by conventional crossing. These criteria are met for the GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

Upon reception of methods, samples and related data (step 1), the EURL GMFF carried out the assessment of the documentation (step 2) and the *in-house* verification of the methods (step 3) according to the requirements of Regulation (EC) 503/2013 (Annex III).

The results of the *in-house* verification study were evaluated with reference to ENGL method performance requirements [3] and to the validation results on the individual events.

2. Step 1 (dossier reception and acceptance)

Monsanto Company submitted the detection methods, data demonstrating their adequate performance when applied to genomic DNA extracted from the stack, and the corresponding control samples of DNA extracted from the GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize and from non GM maize.

The dossier was found to be complete and thus was moved to step 2.

3. Step 2 (dossier scientific assessment)

The data provided by the applicant were assessed against the method acceptance criteria set out by the ENGL[3] and with regard to their documentation and reliability.

Table 1 shows values of trueness (expressed as bias %) and precision (expressed as RSD_r %) calculated by the applicant for the five methods applied to MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize genomic DNA. Means are the average of fifteen replicates obtained through one run (two runs for 1507) performed with ABI7500 real-time PCR equipment. Some results of the reference system, *hmg,* at 1.0 % and 0.085 % levels were evaluated by the applicant as outliers by Grubb's Test for Outliers and excluded from the final evaluation for events MON 87427, MON 89034, 1507, MON 88017 and 59122. In these cases the means are the average of fourteen replicates. Percentages are expressed as GM DNA / total DNA x 100.

Note: Numerical values presented in the following tables were rounded keeping two digits for values ≤ 1, one digit for values between 1 and 10 and no digit for values ≥ 10, unless otherwise stated. The calculations in the MS Excel files however were done over not rounded data. This approach might create small inconsistencies in the numerical values reported in the tables.

Table 1. Trueness (expressed as bias %) and precision (expressed as relative repeatability standard deviation, RSDr %) provided by the applicant for the MON 87427, MON 89034, 1507, MON 88017 and 59122 methods applied to GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

MON 87427*			
Sample GM %	**Expected value (GMO %)**		
	0.085	**1.0**	**10**
Mean	0.094	1.01	11.39
RSD_r (%)	12.52	5.30	7.59
Bias (%)	10.74	0.74	13.93
MON 89034*			
Sample GM %	**Expected value (GMO %)**		
	0.085	**1.0**	**10**
Mean	0.088	1.08	10.62
RSD_r (%)	17.38	5.83	6.22
Bias (%)	3.53	7.86	6.16

1507*				
Sample GM %	**Expected value (GMO %)**			
	0.2	**0.085**	**1.0**	**10**
Mean	0.21	0.071	0.81	8.64
RSD_r (%)	11.37	27.07	6.86	9.47
Bias (%)	0.14	15.91	-18.74	-13.55

MON 88017*			
Sample GM %	**Expected value (GMO %)**		
	0.085	**1.0**	**10**
Mean	0.081	0.99	10.35
RSD_r (%)	10.89	8.27	6.63
Bias (%)	-4.21	-1.06	3.51
59122*			
Sample GM %	**Expected value (GMO %)**		
	0.085	**1.0**	**10**
Mean	0.079	0.92	8.96
RSD_r (%)	14.24	6.96	11.65
Bias (%)	-7.61	-8.12	-10.38

* Numbers are not rounded but are presented as reported by the applicant

The EURL GMFF verified the data and concluded that they were reliable and seemed to confirm that the methods meet the ENGL performance criteria [(3)].

The value of RSDr obtained by the applicant on the sample at 0.085% for event 1507 was slightly above the acceptance limit (27.07 %); the applicant tested the method on an additional sample at 0.2 % GMO which passed all criteria (see table 1).

Three requests of complementary information regarding the method, the control samples and DNA sequences were addressed to the applicant. The EURL GMFF verified the data and the complementary information received and accepted the received clarifications as satisfactory.

The dossier was therefore moved to step 3.

4. Step 3 (EURL GMFF experimental testing)

In step 3 the EURL GMFF implemented the five methods in its own laboratory and performed a verification of their performance when applied to genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

4.1 Materials

The following control samples were provided by the applicant:

- genomic DNA extracted from homogenized seeds of GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize (similar to HCL301 x LH287), hemizygous for the loci, as positive control sample.
- genomic DNA extracted from homogenized seeds of conventional (non GM) maize (HCL301 x LH287), as negative control sample.

The EURL GMFF prepared test samples of different GMO concentrations by mixing genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize with the non GM maize genomic DNA, in a constant amount of total maize genomic DNA. The same GM concentrations as in the validation of the methods for the single lines were achieved. Table 2 shows the five GM concentrations used in the verification of the MON 87427, MON 89034, 1507, MON 88017 and 59122 methods when applying them to genomic DNA extracted from the GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

Table 2. Percentage (GM %) of MON 87427, MON 89034, 1507, MON 88017 and 59122 in MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize stack genomic DNA contained in the verification samples.

MON 87427 GM%* [(GM DNA / total maize DNA x 100)]	**MON 89034 GM%*** [(GM DNA / total maize DNA x 100)]	**1507 GM%*** [(GM DNA / total maize DNA x 100)]	**MON 88017 GM%*** [(GM DNA / total maize DNA x 100)]	**59122 GM%*** [(GM DNA / total maize DNA x 100)]
0.06	0.09	0.1	0.09	0.1
0.2	0.4	0.5	0.5	0.4
0.9	0.9	0.9	0.9	0.9
3.0	3.0	2.0	5.0	2.0
8.0	8.0	5.0	8.0	4.5

* percentages expressed in copy number ratio.

The protocols described by the applicant for the individual MON 87427, MON 89034, 1507, MON 88017 and 59122 GM events (available at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx) were implemented in the EURL GMFF laboratory with the deviations described in §4.4.1.

4.2 DNA extraction

A method for DNA extraction from maize was previously evaluated by the EURL GMFF with regard to its performance characteristics and was considered valid, i.e. fit the purpose of providing maize DNA of appropriate quality and amount for being used in subsequent PCR experiments.

Consequently, the EURL GMFF did not verify the DNA extraction method proposed by the applicant.

The protocol for the DNA extraction method is available at http://gmo-crl.jrc.ec.europa.eu/summaries/MON89034_DNAExtr_report.pdf.

The EURL GMFF recommends that laboratories using this validated method for testing complex or difficult matrices always verify that the extracted genomic DNA is of sufficient quality.

4.3 Experimental design

Eight PCR runs were carried out for each method. In each run, samples were analysed in parallel with both the GM-specific system and maize reference system *high mobility group (hmg)*,. Five GM levels were examined per run, each GM level in duplicate. PCR analysis was performed in triplicate for all samples. In total, for each method MON 87427, MON 89034, 1507, MON 88017 and 59122, the quantification of the five GM levels was performed as an average of sixteen replicates per GM level (8 runs x 2 replicated levels per run). An Excel spreadsheet was used for determination of the GM %.

4.4 PCR methods

During the verification study, the EURL GMFF carried out parallel tests on DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize using the single detection methods previously validated for the respective single GM events MON 87427, MON 89034, 1507, MON 88017 and 59122.

For detection of GM maize events MON 87427, MON 89034, 1507, MON 88017 and 59122, DNA fragments of 95-bp, 77-bp, 58-bp, 95-bp and 86-bp respectively are amplified using specific primers. PCR products are measured during each cycle (real-time) by means of target-specific oligonucleotide probes labelled with two fluorescent dyes: FAM (6-carboxyfluorescein) as reporter dye at their 5′-end and TAMRA (6-carboxytetramethylrhodamine) as a quencher dye at their 3′-end for all events, except for event MON 89034 making use of a probe labelled with MGBNFQ (minor groove binding non-fluorescent quencher).

For quantification of GM maize events MON 87427, MON 89034, 1507, MON 88017 and 59122, a taxon-specific reference system amplifies a 79-bp fragment of *high mobility group (hmg)*, a maize endogenous gene (GenBank AJ131373.1), using two *hmg* gene-specific primers and a gene-specific probe labelled with FAM and TAMRA.

For the relative quantification of GM maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 standard curves are generated for the MON 87427, MON 89034, 1507, MON 88017 and 59122 systems and for the *hmg* specific system by plotting Cq values of the calibration standards against the logarithm of the DNA amount and by fitting a linear regression into these data. Thereafter, the Cq values of the unknown samples are measured and, by means of the regression formula, the relative amount of MON 87427, MON 89034, 1507, MON 88017 and 59122 DNA is estimated.

For detailed information on the preparation of the respective standard curve calibration samples please refer to the protocols of the validated methods at http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx.

4.4.1 Deviations from the validated methods

The *hmg* method validated for the relative quantification of event MON 89034 makes use of TaqMan® buffer A (Life Technologies), which was phased out at the time of method verification by the manufacturer. Therefore, the EURL GMFF replaced the *hmg* method with the *hmg* validated for the relative quantification of maize event MON 87460 (EURL-VL-04/09VP, page 8, at http://gmo-crl.jrc.ec.europa.eu/summaries/2012-01-27_MON87460_validated_Method.pdf); this makes use of the TaqMan® Universal Master Mix (Life Technologies).
For events MON 87427, 1507, MON 88017 and 59122 no deviations were introduced to the original validated methods.

4.5 Results

Tables 3 to 8 present the values of the slopes of the different standard curves generated by the EURL GMFF when using DNA extracted from the GM stack, from which the PCR efficiency is calculated using the formula $[10^{(-1/slope)} - 1] \times 100$, and of the coefficient of determination (R^2) reported for all PCR systems in the eight runs, for GM maize events MON 87427, MON 89034, 1507, MON 88017 and 59122. Slope values were rounded to two digits.

Table 3. Values of standard curve slope, PCR efficiency and R^2 coefficient for the MON 87427 method on GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

Run	MON 87427			*hmg*		
	Slope	**PCR Efficiency (%)**	**R^2 coefficient**	**Slope**	**PCR Efficiency (%)**	**R^2 coefficient**
1	-3.26	103	1.00	-3.24	104	1.00
2	-3.22	105	1.00	-3.19	106	1.00
3	-3.28	102	1.00	-3.23	104	1.00
4	-3.21	105	1.00	-3.21	105	1.00
5	-3.33	100	1.00	-3.23	104	1.00
6	-3.31	100	1.00	-3.25	103	1.00
7	-3.31	100	1.00	-3.16	107	1.00
8	-3.19	106	1.00	-3.19	106	1.00
Mean	-3.26	103	1.00	-3.21	105	1.00

The first tests on the 0.06 % GM sample showed a sub-optimal bias % for the MON 87427 method; the sample was therefore re-prepared and analysed in sixteen replicates in two runs. The values of the slopes of these two additional standard curves, together with the PCR efficiency and the R^2 are presented in Table 4.

Table 4. Values of standard curve slope, PCR efficiency and linearity (R^2) for the MON 87427 method on GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize for the quantification of the re-prepared sample at 0.06 % GM.

Run	MON 87427			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.29	102	0.99	-3.27	102	1.00
2	-3.27	102	1.00	-3.29	101	1.00
Mean	-3.28	102	1.00	-3.28	102	1.00

Table 5. Values of standard curve slope, PCR efficiency and R^2 coefficient for the MON 89034 method on GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

Run	MON 89034			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.56	91	0.99	-3.35	99	1.00
2	-3.52	92	1.00	-3.37	98	1.00
3	-3.40	97	1.00	-3.42	96	1.00
4	-3.47	94	1.00	-3.27	102	1.00
5	-3.45	95	1.00	-3.32	100	1.00
6	-3.44	95	1.00	-3.36	98	1.00
7	-3.45	95	1.00	-3.39	97	1.00
8	-3.45	95	1.00	-3.37	98	1.00
Mean	-3.47	94	1.00	-3.36	99	1.00

Table 6. Values of standard curve slope, PCR efficiency and R^2 coefficient for the 1507 method on GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

Run	1507			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.18	106	1.00	-3.24	104	1.00
2	-3.41	96	0.98	-3.44	95	1.00
3	-3.11	110	1.00	-3.21	105	1.00
4	-3.10	110	0.99	-3.21	105	1.00
5	-3.17	107	0.99	-3.27	102	0.99
6	-3.00	115	0.99	-3.14	108	0.99
7	-3.03	114	0.95	-3.16	107	1.00
8	-3.30	101	0.98	-3.36	98	1.00
Mean	-3.16	107	0.99	-3.25	103	1.00

Table 7. Values of standard curve slope, PCR efficiency and R^2 coefficient for the MON 88017 method on GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

Run	MON 88017			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.39	97	1.00	-3.45	95	1.00
2	-3.37	98	1.00	-3.44	95	1.00
3	-3.33	100	1.00	-3.44	95	1.00
4	-3.50	93	1.00	-3.53	92	1.00
5	-3.41	97	1.00	-3.38	98	1.00
6	-3.37	98	1.00	-3.44	95	1.00
7	-3.52	93	1.00	-3.34	99	1.00
8	-3.38	97	1.00	-3.48	94	1.00
Mean	-3.41	97	1.00	-3.44	95	1.00

Table 8. Values of standard curve slope, PCR efficiency and R^2 coefficient for the 59122 method on GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

Run	59122			*hmg*		
	Slope	PCR Efficiency (%)	R^2 coefficient	Slope	PCR Efficiency (%)	R^2 coefficient
1	-3.42	96	0.99	-3.22	104	1.00
2	-3.27	102	1.00	-3.27	102	1.00
3	-3.30	101	1.00	-3.24	104	1.00
4	-3.41	97	1.00	-3.16	107	1.00
5	-3.25	103	1.00	-3.33	100	1.00
6	-3.34	99	1.00	-3.28	102	0.99
7	-3.22	105	1.00	-3.25	103	0.99
8	-3.42	96	1.00	-3.47	94	0.99
Mean	-3.33	100	1.00	-3.28	102	1.00

The mean PCR efficiencies of the GM and species-specific systems were between -3.47 and -3.16. The mean R^2 coefficient of the methods was 1.00 for all systems in all cases, except for the 1507 system (0.99). The data presented in Tables 3 to 8 confirm the appropriate performance characteristics of the five methods when tested on GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize in terms of PCR efficiency and R^2 coefficient.

The EURL GMFF also assessed the values of trueness (expressed as bias %) and precision (expressed as relative repeatability standard deviation, RSD_r %) of the five methods applied to samples of DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize, see tables 9 to 13.

Table 9. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the MON 87427 method applied to genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

MON 87427					
Unknown sample GM %	**Expected value (GMO %)**				
	0.06*	**0.20**	**0.90**	**3.0**	**8.0**
Mean	0.06	0.16	0.74	2.89	7.74
SD	0.01	0.01	0.03	0.15	0.46
RSD_r (%)	9.9	8.4	4.7	5.1	5.9
Bias (%)	6.1	-19	-18	-3.8	-3.2

*Results on the second preparation of the 0.06 % GM sample

Table 10. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the MON 89034 method applied to genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

MON 89034					
Unknown sample GM %	**Expected value (GMO %)**				
	0.09	**0.40**	**0.90**	**3.0**	**8.0**
Mean	0.09	0.40	0.87	3.0	7.4
SD	0.01	0.03	0.09	0.24	0.71
RSD_r (%)	13	8.4	10	8.1	9.6
Bias (%)	-5.1	0.46	-2.9	-0.18	-8.1

Table 11. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the 1507 method applied to genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

1507					
Unknown sample GM %	**Expected value (GMO %)**				
	0.10	**0.50**	**0.90**	**2.0**	**5.0**
Mean	0.09	0.45	0.85	2.0	5.3
SD	0.02	0.10	0.17	0.35	0.63
RSD_r (%)	23	22	20	18	12
Bias (%)	-11	-9.2	-5.7	-0.40	5.4

Table 12. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the MON 88017 method applied to genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

MON 88017					
Unknown sample GM %	**Expected value (GMO %)**				
	0.09	**0.50**	**0.90**	**5.0**	**8.0**
Mean	0.11	0.58	1.0	5.6	8.8
SD	0.02	0.06	0.07	0.50	0.60
RSD_r (%)	14	9.6	7.1	8.9	6.8
Bias (%)	19	16	12	12	10

Table 13. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the 59122 method applied to genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

59122					
Unknown sample GM %	**Expected value (GMO %)**				
	0.10	**0.40**	**0.90**	**2.0**	**4.5**
Mean	0.10	0.43	0.92	2.1	4.8
SD	0.02	0.08	0.13	0.37	0.75
RSD_r (%)	17	18	15	17	16
Bias (%)	0.12	8.3	2.1	5.9	7.0

The trueness of the method is estimated by measuring the bias for each GM level. According to the ENGL acceptance criteria and method performance requirements, the trueness of the method should be less or equal to ± 25 % across the entire dynamic range. As shown in Tables 9 to 13, the values range from -19 % to 6.1 % for MON 87427, from -8.1 % to 0.46 % for MON 89034, from -11 % to 5.4 % for 1507, from 10 % to 19 % for MON 88017 and from 0.12 % to 8.3 % for 59122. Therefore, the five methods satisfy the above mentioned requirement throughout their respective dynamic ranges, also when applied to DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

Tables 9 to 13 also show the relative repeatability standard deviation (RSD_r) estimated for each GM level. According to the ENGL acceptance criteria and method performance requirements, the RSD_r values should be equal to or below 25%. As the values range between 4.7 % and 9.9 % for MON 87427, between 8.1 % and 13 % for MON 89034, between 12 % and 23 % for 1507, between 6.8 % and 14 % for MON 88017 and between 15 % and 18 % for 59122, the five methods satisfy this requirement throughout their respective dynamic ranges when applied to DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize.

5. Conclusions

The performance of the five event-specific methods for the detection and quantification of single line maize events MON 87427, MON 89034, 1507, MON 88017 and 59122 meets the ENGL performance requirements, when applied to genomic DNA extracted from GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122, as assessed on the control samples provided by the applicant.

Therefore these methods, developed and validated to detect and quantify the single maize events MON 87427, MON 89034, 1507, MON 88017 and 59122, can be equally applied for the detection and quantification of the respective events in DNA extracted from the GM stack MON 87427 x MON 89034 x 1507 x MON 88017 x 59122 maize or any of its sub-combinations, provided that sufficient genomic DNA of appropriate quality is available.

6. References

1. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (Text with EEA relevance). OJ L 268, 18.10.2003, p. 1–23.

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